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Similar Vasoconstrictor Responses to Calcium in Normotensive and Essential Hypertensive Men[1, 2] (38841)

HENRY W. OVERBECK, MOTILAL B. PAMNANI, AND RANDALL S. DERIFIELD

Departments of Physiology and Medicine, Michigan State University, East Lansing, Michigan 48824, and the Veterans Administration Hospital, Saginaw, Michigan

In the absence of calcium, vascular smooth muscle cannot contract. In contrast, increase in the sarcoplasmic concentration of free calcium ion triggers contraction of blood vessels (1). Several investigators have proposed that a defect in the vascular metabolism of this essential cation may underlie the abnormal vasoconstriction of hypertension (1–5). They suggest that this defect may involve increases in cell membrane permeability to the calcium ion. There is *in vitro* evidence that vascular strips from animals with experimental hypertension may be hyperresponsive to calcium; such observations support the hypothesis (3–5).

In vivo, local infusion of calcium into intact vascular beds of dogs evokes vasoconstriction (6–10), probably by increasing sarcoplasmic concentration of free calcium ion. If cell membrane permeability to calcium is, in fact, increased in hypertension, one would expect to also observe vascular hyperresponsiveness to calcium *in vivo*. However, we found no evidence for such abnormal vascular responses to calcium in dogs with renal hypertension (10).

In the present work we investigated local responses to calcium in the limb vascular bed of normotensive men and men with essential hypertension. To our knowledge this is the first such study in man.

Materials and Methods. We studied limb vascular responses in 18 male inpatients at the Veterans Administration Hospital, Saginaw, MI. Ten patients (nine Caucasian, one Black) had normotension documented by several normal casual blood pressure measurements during hospitalization. These convalescing patients had been hospitalized for mild chronic diseases (depression, duodenal ulcer, alcoholism, arthritis, diabetes mellitus) not felt to affect vascular responses, and, at the time of our study, were afebrile and not receiving vasoactive drugs.

The other eight patients (five Caucasian, three Black) had essential hypertension of mild to moderate severity documented by thorough study, including hospital diastolic blood pressures averaging above 90 mm Hg during hospital days 4–6, normal rapid sequence intravenous pyelograms, normal 24-hr urine vanilmandelic acid (VMA) excretion and normal serum sodium, potassium, and calcium concentrations. Severity indices in hypertensives were calculated according to the criteria of the Veterans Administration Cooperative Study (11). No hypertensives had retinal hemorrhages, exudates, or papilledema. All antihypertensive or vasoactive drugs and diets were discontinued at least 4 wk before the response study. No subjects had clinically discernible cardiac insufficiency, elevated serum creatinine or blood urea nitrogen concentrations, or significant proteinuria.

All subjects participating were fully informed by the investigators of the purposes, procedures, and hazards of the experiment; written consent was obtained. Procedures used were in accordance with institutional policies.

These volunteers were studied in the resting, postabsorptive state, and supine position with laboratory temperature ranging from 29 to 30°. The procedures employed for infusions and measurement of limb intravascular pressures and calculation of blood flows by indicator-dilution have been described in detail (12, 13). Briefly, we used a jet-injection system to infuse solutions intrabrachial-arterially at 8.2 ml/min and at 37°. All solutions contained indicator, approximately

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0.06 μCi ^{131}I per ml (^{131}I-labeled human serum albumin in isotonic sodium chloride solution, Albumotope, Squibb, New Brunswick, NJ, or IHSA I 131, Mallinckrodt Chemical Works, St. Louis, MO). The experimental solutions also contained isosmolar (285 mOsm/kg) $CaCl_2$. Control and experimental solutions were brought to volume with isosmolar NaCl solution. Infused at 8.2 ml/min, the solutions containing $CaCl_2$ delivered 0.115, 0.230, or 0.460 meq calcium/min into brachial arterial blood. For a blood flow of 100 ml/min it may be calculated that these infusions would increase limb arterial plasma calcium concentration by 2.1, 4.2, or 8.4 meq/liter, respectively. Three sets of paired infusions were made into each subject: each set included first a control infusion, then the experimental infusion containing $CaCl_2$. Thus, the effect of the infusion of $CaCl_2$ was compared to "baseline" hemodynamics measured during the paired control infusion. The solutions containing $CaCl_2$ were always administered in order of increasing dose. Each infusion lasted about 16 min.

During infusions we sampled ipsilateral cephalic venous and basilic venous and contralateral brachial arterial blood simultaneously at the 10th and 15th minute of each infusion for determination of hematocrit and of ^{131}I concentrations. We calculated blood flow from these isotope concentrations. We also recorded pressures in the ipsilateral cephalic and basilic veins and contralateral brachial artery in turn immediately after we sampled blood for ^{131}I concentrations. The equations for calculating limb blood flow and vascular resistance have been presented (13). Blood flows and resistances were calculated on a per limb volume basis. All experiments reported were considered technically satisfactory, because they met our defined criteria (13) for adequate mixing of infusate with limb blood, restoration of steady-state blood flow between vasoactive infusions, and subject comfort and cooperation.

In nine normotensive and seven hypertensive subjects serum sodium and potassium concentrations and osmolality of the cephalic venous and contralateral arterial samples taken at the 10th minute of the infusion were measured as previously described (13). Serum calcium and magnesium concentrations were measured on a Perkin–Elmer Atomic Absorption Spectrometer (Model 290).

For data analysis we used the paired Student's *t* test (14) to compare responses to control isosmolar NaCl solution with those to the paired isosmolar $CaCl_2$ solution, and to compare serum electrolyte concentrations and osmolalities during saline and $CaCl_2$ infusions. The unpaired *t* test was used to compare pressures, flows, resistances, responses, and concentrations in normotensive patients with values in hypertensive patients. Linear correlation and regression coefficients were also calculated for each dose level of $CaCl_2$ to determine if there were significant relationships between limb initial resistance (resistance during the paired control isosmolar NaCl infusion) and magnitude of response to $CaCl_2$. We then used analysis of covariance to compare regression-adjusted responses in hypertensive patients with those in normotensive patients.

Results. We completed studies in 10 normotensive and eight hypertensive subjects. Clinical data on these subjects are presented in Table I. Between the two groups there were no significant differences in ages, body weights, limb volumes, blood hematocrits, blood urea nitrogens, serum potassium, sodium, magnesium, calcium, and albumin concentrations. More hypertensives than normotensives were Black. We classified hypertensive disease in 75% of the essential hypertensive patients as of moderate severity and in the remainder as mild.

The intrabrachial arterial infusions altered

TABLE I. SUBJECTS.[a]

	Normotensives (10)	Hypertensives (8)
Age (yr)	49.1 ± 2.7	51.1 ± 2.1
Body wt (kg)	80.7 ± 2.6	87.2 ± 6.5
Limb vol (cc)	1669 ± 64	1762 ± 112
Hct (vol/100 ml)	43.5 ± 1.0	42.4 ± 0.8
Serum calcium (meq/liter)	4.8 ± 0.1	4.8 ± 0.1
Serum albumin (g/100 ml)	4.2 ± 0.1	4.2 ± 0.2

[a] Means ± SEM.

TABLE II. LIMB ARTERIAL AND VENOUS CONCENTRATIONS.[a]

		Venous			
			Calcium infusion (meq/min)		
	Arterial	Saline infusion	0.115	0.230	0.460
$[Ca^{2+}]$, meq/liter	4.7 ± 0.1 (32)	4.5 ± 0.1 (32)	—	6.7 ± 0.3** (16)	11.5 ± 0.8** (16)
$[K^+]$, meq/liter	4.1 ± 0.1 (32)	3.7 ± 0.1 (32)	—	3.7 ± 0.1 (16)	3.5 ± 0.2 (15)
$[Na^+]$, meq/liter	145.5 ± 0.9 (32)	146.4 ± 1.0 (32)	—	147.3 ± 1.8 (16)	144.1 ± 2.0 (15)
$[Mg^{2+}]$, meq/liter	1.96 ± 0.04 (32)	1.79 ± 0.05 (32)	—	1.79 ± 0.06 (16)	1.72 ± 0.06 (15)
Hct, vol/100 ml	41.6 ± 0.3 (102)	38.9 ± 0.3 (102)	39.0 ± 0.5 (34)	37.9 ± 0.5 (34)	35.9 ± 0.7** (33)

[a] Mean ± SEM; number of separate determinations indicated in parentheses. Samples obtained from normotensive and hypertensive patients.

** $P < 0.01$, for comparison of limb venous concentration during infusion of isosmolar $CaCl_2$ solution with that during infusion of control isosmolar NaCl solution.

the hematocrit and electrolyte composition of ipsilateral limb venous blood without significantly changing systemic arterial blood composition. Changes induced in limb venous composition are indicated in Table II. Relative to arterial values, the 8.2 ml/min control saline infusions reduced ($P < 0.05$) limb venous hematocrit and serum concentrations of potassium, calcium, and magnesium, without significantly changing limb venous serum sodium concentration or plasma osmolality. As compared to venous serum concentrations during these control NaCl infusions, infusions of isosmolar $CaCl_2$ at 0.230 meq calcium/min increased mean limb venous serum calcium concentrations by 2.2 meq/liter (range 0–4.0) ($P < 0.01$). Range of resulting venous serum concentrations was 4.4–8.6 meq/liter (resulting concentrations were inversely related to limb blood flow). Infusion of isosmolar $CaCl_2$ at 0.460 meq calcium/min increased mean limb venous serum calcium concentrations by 7.0 meq/liter (range 2.4–15.4) ($P < 0.01$). Range of resulting venous serum concentrations was 7.5 to 20.0 meq/liter. In contrast, limb venous serum concentrations of potassium, sodium, magnesium, and osmolality during $CaCl_2$ infusions did not significantly differ from those during control NaCl infusions. Infusion of 0.460 meq calcium/min did, however, reduce limb venous hematocrit, as compared to venous hematocrit during the control saline infusion, by decreasing limb blood flow and thereby increasing the dilutional effect of the infusion. During neither NaCl or $CaCl_2$ infusions did venous concentrations or hematocrits in normotensives differ significantly from those in hypertensives.

There were highly significant ($P < 0.001$) differences between the mean arterial pressures of the two groups directly measured at the time of the response study (Table III). Limb blood flows and resistances in the two groups did not significantly differ, although there was a trend toward higher blood flow and resistance in the hypertensives (in an enlarged study that included some of these same patients we found significantly elevated limb resistances in the hypertensives, but there were still no significant differences in blood flow (15)). The effects of the intrabrachial arterial infusions are also presented in Table III. The calcium infusions had no significant effect on systemic arterial or venous pressures in either group of subjects. In contrast, in the normotensive patients infusion of $CaCl_2$ at each dose level decreased limb blood flow and increased vascular resistance. In the hypertensive patients 0.230 and 0.460 meq calcium/min decreased blood flow and 0.230 meq calcium/min increased resistance. In hypertensives resistance increases in response to 0.460 meq calcium/min were of borderline statistical significance ($P < 0.06$). Resistance during the following control saline infusions returned to or toward the original baseline levels. In hypertensives decreases in limb blood flows and

TABLE III. LIMB HEMODYNAMIC RESPONSES TO INTRAARTERIAL INFUSIONS.[a]

Infusion	meq Ca^{2+}/min	Limb blood flow (ml/min/100 cc)	Change in limb blood flow	$\bar{P}_A$ (mm Hg)	Vascular resistance mm Hg/ml/min/100 cc)	Change in resistance
Normotensives (N = 10)						
Control	0.000	5.97 ± 0.54		89.3 ± 2.4	14.63 ± 1.79	
$CaCl_2$	0.115	5.35 ± 0.51	−0.62 ± 0.27*	89.6 ± 2.4	16.40 ± 1.89	+1.77 ± 0.74*
Control	0.000	6.29 ± 0.75		89.4 ± 2.7	16.04 ± 3.50	
$CaCl_2$	0.230	4.36 ± 0.57	−1.92 ± 0.37**	90.6 ± 2.6	23.02 ± 3.99	+6.98 ± 1.69**
Control	0.000	5.29 ± 0.62		92.8 ± 2.3	18.36 ± 2.74	
$CaCl_2$	0.460	3.11 ± 0.47	−2.19 ± 0.31**	94.6 ± 2.5	33.73 ± 5.39	+15.37 ± 3.71**
Hypertensives (N = 8)						
Control	0.000	6.57 ± 0.64		117.1 ± 4.4	18.04 ± 3.06	
$CaCl_2$	0.115	6.08 ± 0.68	−0.49 ± 0.32	116.9 ± 4.3	20.35 ± 3.96	+2.31 ± 1.74
Control	0.000	6.88 ± 0.71		117.1 ± 4.3	17.40 ± 2.88	
$CaCl_2$	0.230	4.61 ± 0.54	−2.27 ± 0.40**	117.6 ± 4.3	25.86 ± 3.71	+8.46 ± 2.14**
Control	0.000	5.88 ± 0.98		118.5 ± 4.2	22.61 ± 3.40	
$CaCl_2$	0.460	3.84 ± 0.73	−2.04 ± 0.63*	119.5 ± 3.9	41.94 ± 10.63	+19.32 ± 8.30

[a] Means ± SEM. $\bar{P}_A$ = mean brachial arterial pressure.
* $P < 0.05$; ** $P < 0.01$. Significance values are for comparison of variables during control infusion of isosmolar NaCl with those during infusion of isosmolar $CaCl_2$.

increases in vascular resistance in response to calcium were not significantly different from corresponding values in normotensives ($P >$ 0.8 at each dose level, by Student's t test).

In many subjects, during the $CaCl_2$ infusions, we noted blanching of ipsilateral forearm and hand skin which disappeared during the following control infusion. During the calcium infusions most normotensive and hypertensive subjects noted mild but not uncomfortable sensations in that hand. They described these sensations as "warmth," "heat," "burning," and/or "numbness." In one normotensive patient, during infusion of 0.460 meq calcium/min, fasciculations developed in forearm muscles which gradually disappeared over the 30 min after the infusion was discontinued (in this subject measured limb venous serum calcium concentrations did not exceed 9.6 meq/liter). Fasciculations or muscle contractions were not noted in other subjects.

Because there is evidence that the magnitude of vascular response (ΔR) to calcium is a function of the level of limb "baseline" or "initial" resistance (IR)(10), we calculated linear correlation coefficients for IR vs ΔR. For infusions of 0.115, 0.230, and 0.460 meq calcium/min these correlation coefficients were 0.212 ($P > 0.05$), 0.077 ($P > 0.05$), and 0.495 ($P < 0.05$), respectively, in the 18 subjects. Therefore, we additionally compared responses in hypertensives and normotensives to 0.460 meq calcium/min by using analysis of covariance, which adjusted responses for their significant regression on initial resistance. Analysis of covariance also provided no evidence that responses in hypertensives were different from those in normotensives ($F < 0.008$; $P > 0.8$).

Finally, Fig. 1 represents log dose-response curves for normotensives and hypertensives constructed from response means and SEM's presented in Table III.

Discussion. The results of the present study indicate that in man acute local elevations in limb plasma calcium concentrations increase limb vascular resistance in a dose-related manner. This increase in resistance occurs in response to increments in calcium ranging from less than 2 up to 20 meq/liter. The vascular effects of calcium are prominent, increases in limb serum calcium concentration averaging as little as 2.2 meq/liter elevating limb resistance by 44% to 49% in the normotensive and hypertensive patients. Thus, such increases in resistance probably play a role in the hypertension accompanying hypercalcemic disease states (16) and in the elevation of blood pressure produced by infusing calcium solutions intravenously into man (17, 18).

There is evidence that blood viscosity is not elevated by such calcium increments (19). Furthermore, in the present study the calcium infusions did not increase limb blood

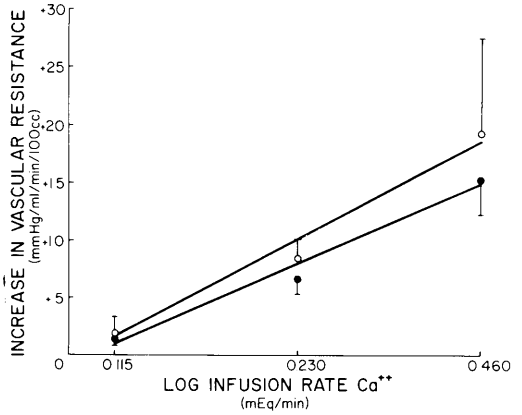

FIG. 1. Log dose-response curves for intrabrachial arterial infusions of isosmolar $CaCl_2$ solution. Constructed from means and SEM's presented in Table III. ● and ○ indicate responses in normotensive and essential hypertensive subjects, respectively.

hematocrit. Therefore, we attribute the increased limb resistance to vasoconstriction. This limb vasoconstriction is a local effect, because the calcium infusions change neither systemic serum calcium concentration nor systemic blood pressures. Further, the vasoconstriction evoked by the calcium infusion is probably not an effect of induced changes in other vasoactive cations in limb blood, because serum concentrations of potassium, sodium, magnesium, and osmolality are not altered relative to concentrations during the control infusion.

These vasoactive effects in man of intra-arterially administered calcium solutions have not, to our knowledge, been previously reported. In dogs, however, several observers have documented the vasoconstrictor properties of intraarterially infused calcium, raising local plasma calcium concentrations by as little as 2 meq/liter. In the dog, calcium vasoconstriction has been observed in various vascular beds, including the limb (6, 7, 9, 10), the kidney (9), and the heart (8). The mechanism of this vasoconstriction is unknown, but is likely the result of gradient-induced increases in sarcoplasmic free calcium ion concentrations (20).

In contrast to these prominent effects of calcium on resistance vessels *in vivo*, relaxed arterial tissue *in vitro* may have little response to large increments in bath calcium concentrations (5, 20). Increasing bath calcium concentrations up to 16 meq/liter may evoke no contraction at all in small limb arteries of dogs (21). Although arterial tissue from normal rats is also relatively unresponsive, increasing bath calcium from 3.2 up to 6.4 meq/liter evokes prominent increases in tension in femoral artery strips from rats with DCA hypertension (4). On the basis of this observation, Bohr and co-workers suggested that the increased responsiveness in hypertensives might be attributable to increased vascular smooth muscle membrane permeability to Ca^{2+}. Tobian and Chesley (22) found increased calcium content in arteriolar walls of one-kidney Goldblatt hypertensive rats and suggested that there may be underlying increased calcium influxes into vascular smooth muscle cells of hypertensives. Similarly, Hinke (3) found increased calcium content in segments of the ventral tail artery from DCA-hypertensive rats. Norepinephrine or high K^+ contraction of these hypertensive arteries was more difficult to abolish during zero calcium perfusion, and more easily reestablished by the addition of calcium to the bath. Hinke's observations have been regarded by Somlyo and Somlyo (1) as additional evidence suggesting increased vascular membrane permeability to calcium in hypertensive animals. From these several *in vitro* studies the evidence suggesting abnormal vascular calcium metabolism in hypertensives is, therefore, quite persuasive.

However, we would reasonably anticipate similar hyperresponsiveness to calcium *in vivo* as well as *in vitro*, if increased permeability to Ca^{2+} plays a significant role in hypertension in life. In this regard, we found no evidence of enhanced responses in limb vascular beds of one-kidney perinephritic hypertensive dogs to infusions producing increases in plasma calcium concentrations within (and above) ranges seen in life (10). Furthermore, in the present study there was no evidence to suggest vascular hyperresponsiveness to calcium in essential hypertensive men (even on the basis of adaptive structural changes increasing vessel wall-to-lumen ratio [23]). We additionally found no differences in limb vascular responses to calcium infusions in normotensive rats, two-kidney Gold-

blatt hypertensive rats, and genetic (New Zealand strain) hypertensive rats (24). The limb vascular bed contains both muscle and skin arterioles; thus we have not excluded the possibility that the arterioles of either component alone may have altered responses to calcium, or that arterioles in vascular beds other than those of the limb may have altered responses. Nevertheless, our several *in vivo* results appear to us not to support the hypothesis, based on *in vitro* observations, that increases in permeability of the membranes of vascular smooth muscle cells to the calcium ion may underlie hypertension, at least in renal hypertensive animals and essential hypertensive men.

In previous experiments in some of the same essential hypertensive men (15) and also in our studies of renal hypertensive dogs (10) and rats (25), we have noted consistently attenuated vasodilator responses to the potassium ion. In view of the apparently normal responses of essential hypertensive men to calcium, and to magnesium in a previous study (12), the abnormality in response to K^+ in essential hypertensives appears to be specific. Our present experiments, therefore, add further support to our hypothesis that in hypertension there may be an underlying defect in metabolism of the potassium ion by vascular smooth muscle cells. We have suggested (10, 15) that this defect may involve the cellular Na^+-K^+ activated ATPase system and/or the operation of the electrogenic pump.

Summary. To study limb vascular responses in man to elevations in plasma calcium concentrations, we infused test isosmolar solutions of $CaCl_2$ (0.115, 0.230, and 0.460 meq calcium/min) and NaCl and control isosmolar solutions of NaCl into the brachial arteries of 10 normotensive men and eight men with essential hypertension of mild to moderate severity. Limb blood pressures were monitored, limb blood flow was measured by indicator-dilution, and limb vascular resistance was calculated as mm Hg/ml flow/min/100 cm^3 limb volume. Measured concentration of calcium in limb venous plasma during infusion of 0.460 meq calcium/min was 11.5 ± 0.8 meq/liter (mean ± SEM) with individual values ranging up to 20 meq/liter. Changes in limb venous serum sodium, potassium, magnesium, and osmolality were similar during control and $CaCl_2$ infusions. Decreases in limb venous blood hematocrit during $CaCl_2$ infusions were the same or greater than those during control infusions. The infusions did not significantly change systemic blood calcium concentration or blood pressures. Limb blood flow decreased and resistance increased in response to $CaCl_2$. Increments averaging as little as 2.2 meq/liter elevated limb resistance by about 45%. Log dose-response curves were linear. Responses did not differ in normotensives and hypertensives ($P > 0.8$). We conclude that the vascular response to acute elevation of plasma calcium concentrations up to 20 meq/liter in the limb of man is an impressive vasoconstriction. We found no evidence for abnormal vascular responses to calcium in essential hypertensive men.

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